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PB-265 311

Larval Feeding and Rapid Maturation of Bluegills in the Laboratory

Environmental Research Lab -Duluth, Minn

1976

TECHNICAL REPORT DATA
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1. REPORT NO.	2.	3. PB 265 311
EPA-600/J-76-025	JOURNAL ARTICLE	

4. TITLE AND SUBTITLE	5. REPORT DATE
LARVAL FEEDING AND RAPID MATURATION OF BLUEGILLS IN THE LABORATORY *	April 1976
	6. PERFORMING ORGANIZATION CODE: EPL-DUL-080
7. AUTHOR(S): Wesley E. Smith	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Environmental Research Laboratory Office of Research and Development U.S. Environmental Protection Agency Duluth, Minnesota 55804	10. PROGRAM ELEMENT NO.: 1BA608 11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS Environmental Research Laboratory Office of Research and Development U.S. Environmental Protection Agency Duluth, Minnesota 55804	13. TYPE OF REPORT AND PERIOD COVERED: Journal Article 14. SPONSORING AGENCY CODE: EPA/600/03

15. SUPPLEMENTARY NOTES: CONTACT: W.E. Smith, 8-783-9532 (Duluth, MN)
* as published in Progressive Fish-Culturist, 38(2): 95-97.

16. ABSTRACT

Bluegill larvae were raised in the laboratory with a high percentage of survival using newly hatched San Francisco brine shrimp nauplii as a first food. Utah brine shrimp nauplii and older San Francisco nauplii were too large for a bluegill first food. Bluegills were raised to maturity in 4-6 months.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Freshwater fishes Aquatic biology Feeding stuff Maturation	Bluegills Larval fish Larval feeding Growth rates	06C

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	5
	20. SECURITY CLASS (This page)	22. PRICE PC A02 MF A01
	UNCLASSIFIED	

EPA Form 2220-1 (9-73)

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EPA-600/J-76-025
JOURNAL ARTICLE

Larval Feeding and Rapid Maturation of Bluegills in the Laboratory

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The bluegill (*Lepomis macrochirus*) has been used as a bioassay test species by a number of investigators. At the Environmental Research Laboratory — Duluth, Duluth, Minnesota, fish are often tested at each stage of their life cycle. Test criteria include reproductive ability, percentage batch of embryos, and percentage survival of fry. Two problems encountered by investigators working with bluegills are an inability to rear a reasonable percentage of the larvae and the excessive time required for the fish to reach maturity.

The purposes of this study were to develop techniques for obtaining a high percentage survival of larval bluegills and to determine if time to maturity could be shortened by cultural methods.

The literature on the use of bluegills in the laboratory is relatively abundant, but few authors mention percentage survival of larvae beyond the critical stage Eaton (1970) recorded bluegill fry survival at 90 days as 10-20%. He attributed larval death to starvation. In a later investigation Eaton (1974) obtained higher survival of bluegill larvae by twice-daily feedings of laboratory-reared plankton from mixed cultures of zooplankton and phytoplankton; under this treatment larvae had a survival rate of 78% at 60 days. Banner and VanArman (1973) tested the survival of bluegill larvae and compared the results of feeding larvae *Artemia* only and of feeding a mixed diet of rotifers, limnoplankton, and *Artemia*. The larvae fed on the mixed diet died within 2 days, whereas larvae fed only *Artemia* had a survival rate of more than 90% after 8 days; at 15 days, however, the maximum larval survival was 46% (the variety of brine shrimp used was not mentioned). Personal communications with many investigators who have worked with bluegills revealed similar difficulty in carrying the larval fish successfully through a period of about 1 wk after the start of active feeding.

In a study of larval bluegill nutrition Toetz (1966) defined the critical period as beginning 4.4 days after hatching (at the onset of feeding) and ending at 8.5-9 days (the time of 50% larval mortality when starved). He also reported that the mouth gapes of larval bluegills 4 and 9 days after hatching measured 0.20 and 0.33 mm, respectively. He gave a mean length of brine shrimp nauplii as 0.47 mm and deduced that they are too large to be ingested by bluegill larvae.

Negative results with brine shrimp nauplii as a food for bluegill larvae have been reported to me by other investigators, and these comments seem to be substantiated in the literature (Toetz 1966). Two varieties of brine shrimp eggs are generally available: one from the San Francisco Bay area in California and one from salt flats in Utah. Although the "San Francisco nauplii" are known to be smaller than the "Utah nauplii," a search of the literature revealed no measurements of the two varieties. Investigators using brine shrimp nauplii as a fish food have rarely specified the area from which the nauplii came. Furthermore, the influence of the age of nauplii on their size has apparently not been considered.

No known attempt has been made in the laboratory to accelerate the development of bluegills to sexual maturity. Spawning occurs at age-1 in pond culture (Davis 1965); however, Swingle and Smith (1950) reported that in Alabama, under extremely favorable conditions, bluegills spawn as early as 4 mo after hatching. Investigators who have worked with bluegills in the laboratory report times to maturity ranging from about 1 yr to almost 3 yr (personal communications).

Materials and Methods

Preliminary Test

A 21-day preliminary test was made to determine the effect of fungicide treatment on the eggs and to evaluate the comparative worth of several foods for the larvae.

Adult bluegills were spawned in the laboratory, and 100 embryos were incubated at 25°C in each of 10 embryo cups placed on a continuous rocker (Mount 1968). Five cups containing newly collected eggs were given a 5-min dip in 4 mg/l malachite green before placement on the rocker; the other five batches of eggs were placed on the rocker without treatment. When the embryos hatched, 50 larval fish were placed in each of 10 60-liter glass rearing tanks. Water delivery to the tanks was by proportional diluter (Mount and Brungs 1967), which gave a water turnover of 2.4 tank volumes

The Progressive Fish-Culturist, Vol. 38, No. 2, 95-97, April 1976.

per day per tank. Food was first introduced on the third day after hatching when the larvae had begun to dart upward from the tank bottoms but were not yet free-swimming and had not yet absorbed their yolk sacs. Foods provided were green algae, egg yolk suspension, cultured Infusoria, and a dry powdered fish-fry food (Tetra Minn E). Each food was given to 100 larvae in each of six duplicate tanks hourly from 0800 to 1600 h and once or twice during the evening. An attempt was made to keep food available to the larvae at all times during the 16-h light period. On day 6 after hatching, San Francisco brine shrimp nauplii were fed hourly (as above) to fish in all tanks. The original foods were also given for 2 more days, but from day 8 the fish received nothing but the brine shrimp nauplii. The test was terminated 21 days after hatch.

Five 21-day-old bluegills from the preliminary feeding study were placed in each of four 200-liter glass aquariums with slate bottoms. Water temperature was maintained by a head-box heat-exchanger thermostatic control system at 28+1°C. Lake Superior water was delivered to each tank at a rate of 200 ml/min. Photoperiod was regulated by automatic timer and maintained at a constant 16 h of light.

Fish in each tank received *Artemia* nauplii 8 times daily until they were 30 days old. Frozen adult brine shrimp were then given as the only food at the rate of 8 times daily until maturity when feeding was decreased to 4 times daily. Food was supplied in excess to insure that the less aggressive fish, as well as the dominant individuals, were amply fed. Uneaten food was removed once daily by siphoning. After each spawning 100 eggs were collected and the embryos hatched as before.

Other Feeding Tests

The preliminary test demonstrated that bluegill larvae could be raised in the laboratory with a high rate of survival, but it left several questions unanswered. A second test was made to specifically determine whether it was necessary to provide the larvae with initial feedings of material other than brine shrimp nauplii, or whether survival of larvae would be adequate if nauplii were used as a first food. The second test was like the preliminary study except that another pair of tanks was added and feeding was altered as summarized in Table 1.

Size of Nauplii

San Francisco and Utah brine shrimp nauplii were measured twice — 4 and 48 h after hatching — with a binocular dissecting microscope fitted with an ocular micrometer (Table 2).

[1] Mention of commercial products does not constitute endorsement by the U.S. Environmental Protection Agency.

Table 1. Percentage survival of bluegill larvae at 21 days after being fed various foods (feeding test No. 2).[a]

Tank designation	Food	Replicate 1	Replicate 2
A	Powdered: San Francisco nauplii after day 6	94	95
B	Utah nauplii (2-4 h old)	0	0
C	San Francisco nauplii (2-4 h old	90	93
D	San Francisco nauplii (48 h old	38	44
E	None	0	0

[a] One hundred larvae per tank.

Table 2. Measurements (in micrometers) of nauplii of San Francisco and Utah brine shrimp.

Source of brine shrimp, and age (h)	Length (μm) Mean	Length (μm) Range	Mean width (μm)
Utah			
4	509	429-627	166
48	678	594-746	179
San Francisco			
4	394	363-445	152
48	533	429-627	158

Results and Discussion

A 5-min dip in 4 mg/l malachite green apparently increased the percentage hatch of bluegills. The hatch ranged from 88 to 96% for treated eggs and from 47 to 73% for untreated ones.

In the preliminary test larvae thrived in all eight tanks; percentage survival was high with all foods tested. Mean survival of larvae ranged from 91% for egg yolk to 99% for dry food.

Measurements of the brine shrimp nauplii showed that (1) San Francisco nauplii are smaller than Utah nauplii; (2) both varieties of nauplii are smaller 4 h after hatching than they are when 2 days old; and (3) within any of the groupings there is a substantial range in size (Table 2). These data may explain previous failures to rear bluegill larvae on brine shrimp nauplii, for they show important differences in size with age and variety.

In the second larval feeding test, larvae began feeding on San Francisco nauplii late on day 4 after hatching. On day 6 a 50% mortality of larvae occurred in tanks given Utah nauplii and in those given nothing; no fish died in the other tanks. By day 8, all larvae that had been given Utah nauplii and those fed nothing had died, presumably from starvation; a microscopic ex-

amination of the guts of these larvae showed no food material. In contrast, the larvae given San Francisco nauplii and those started on dry food and changed to San Francisco nauplii on day 6 after hatching were feeding well, with abdomens visibly enlarged and orange due to ingested nauplii. Mean survival rates at 21 days were 94.5% for larvae given dry food followed by nauplii and 91.5% for larvae started on San Francisco nauplii (Table 1).

These tests demonstrate that San Francisco brine shrimp nauplii, given shortly after hatching of the shrimp, are an adequate first food for bluegill larvae and that the larvae so fed have a high survival rate. They likewise illustrate the ineffectiveness of Utah nauplii and older San Francisco nauplii, which are apparently too large for a larval first food.

Bluegills in the maturity study first spawned at 4-6 mo. Fish in three of the four tanks spawned a second time 7-10 days after the first spawning. No fish spawned a third time. First spawnings were during the 16th, 17th, 21st, and 24th weeks.

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